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13. ABSTRACT (Maximum 200 Words)

Prostate cancer is the second leading cause of cancer death among men in the western world. Androgen plays a crucial role in the development and growth of normal prostate gland and prostate cancer. Action of androgen is mediated by an androgen receptor (AR) and the AR exerts androgen-regulated gene expression. Standard therapy relies on androgen ablation to remove or block the action of androgens. This therapy results in a regression of the tumor because most primary tumor cells depend on androgens for growth and programmed cell death. However, most prostate cancers eventually relapse as their tumors progress to androgen-refractory. Studies have indicated that the AR gene amplification and mutations are involved in androgen-refractory tumors. Therefore, blockage of the AR gene expression may provide a new approach to the management of the AR-dependent cancer. We have developed anti-AR RNA enzymes that are able to selectively and specially interact with the AR mRNA and cleave the AR mRNA *in vitro*. Unlike conventional chemotherapy, the enzymes would have lesser side effects because the compounds selectively destroy only the AR gene. This study proposed is to determine specific efficacy of these enzymes *in vivo*.

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Introduction

Androgen is essential for the growth of both normal prostate gland and prostate cancer. Action of androgen is mediated by androgen receptor (AR), a member of the nuclear/steroid transcription factors. Although prostate cancer is initially sensitive to androgen ablation therapy, the disease eventually progresses as androgen-refractory. The patients with the androgen-refractory cancers can no longer be cured by any type of conventional therapy. Although the molecular mechanisms of this disease progression remain unknown, studies have shown that AR gene amplification and mutations occur in androgen-refractory tumor specimens of almost all patients, but these evidences are rarely seen in initial cancer. Thus, AR gene amplification and mutations contribute to disease progress. We initially developed short anti-AR RNA enzymes (about 20-40 bases) and these enzymes are able to specially bind to AR mRNA and efficiently catalyze the mRNA, resulting in inhibition of androgen-responsive gene expression *in vitro* and *in cultured cell systems.* The same result was further studied by another laboratory that these RNA enzymes catalyzed AR mRNA and decreased gene expression of prostate specific antigen (PSA) in human androgen-sensitive (LNCaP) and androgen-refractory (LNCaP-C4) cells, and inhibited growth and proliferation of these tumor cells. To test the efficacy of these enzymes on the prostate cancer *in vivo*, we firstly establish two mouse models for this study. The human androgen-sensitive and androgen-refractory tumor cells are microinjected into the prostate glands of the nude mice, respectively. In order to visualize the growth or metastasis of the tumor cells in the nude mice, we have transfected green fluorescent protein (GFP) vector into the two tumor cells. GFP is expressed in both androgen-sensitive and androgen-refractory cells. We selected the GFP as a reporter marker because it is used as a detection tool for cell sorting, measurement of tumor growth in living cells or tissues and not toxic to host cells. Secondly, we have synthesized and delivered these anti-AR RNAs into these tumor cells using different methods. Dynamic interaction of the enzymes with the targeted tumor cells in intracellular movement are observed by the fluorescent imaging system. We will also test effects of these enzymes on the tumor growth or metastasis in the androgen-sensitive and androgen-refractory tumor cells grown as xenografts in the nude mice compared to that of the control group. At different time points, the tumor size or metastasis in the treated and control groups will be measured. Expression levels of AR and PSA as well as cell apoptosis from tissue samples will be detected after the animals are sacrificed.

Body

Androgen is the most important endocrine factor regulating the development and growth of prostate gland and the action of androgens is mediated by the androgen receptor (AR), which is a ligand–responsive transcription factor (1). The AR exerts its action by binding to the androgen response element, to control expression of androgen-regulated genes (2). Overexpression of the AR in mouse prostate gland induced aged-AR transgenic mice to develop intraepithelial dysplasias similar to human prostate cancer (3). Initial prostate cancer is effectively treated with androgen ablation. However, tumor eventually becomes refractory to anti-androgen treatment (4, 5). Although the molecular events of progression from androgen-sensitive to androgen-refractory tumor are unclear, several studies have showed that this progress is associated with AR gene amplification and mutations (6-8). Current therapies are not effective in the control of androgen-refractory prostate cancer (9). Inhibition of AR expression in the tumor cells may provide a way to control disease progress. We initially developed small anti-AR RNA enzymes that are able to specifically and efficiently catalyze AR messenger RNA in vitro (10). Based on these results, we have studied whether these anti-AR RNA enzymes have biological functions in vivo in this proposal. At first, we have established two types of human prostate cancer cell lines, androgen-sensitive (LNCaP) and androgen-refractory (LNCaP-C4), that express green fluorescent protein (GFP) (**Figure 1**).

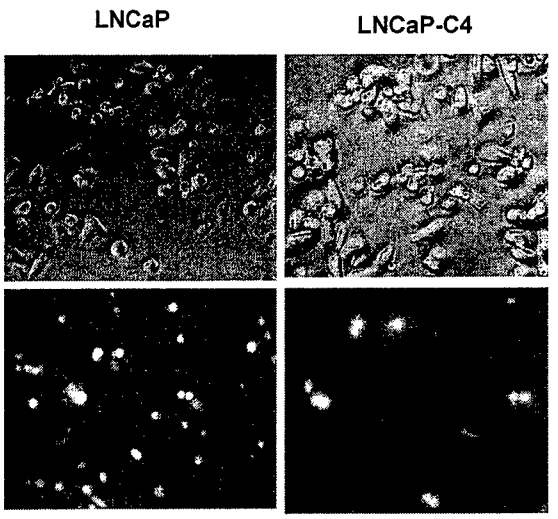
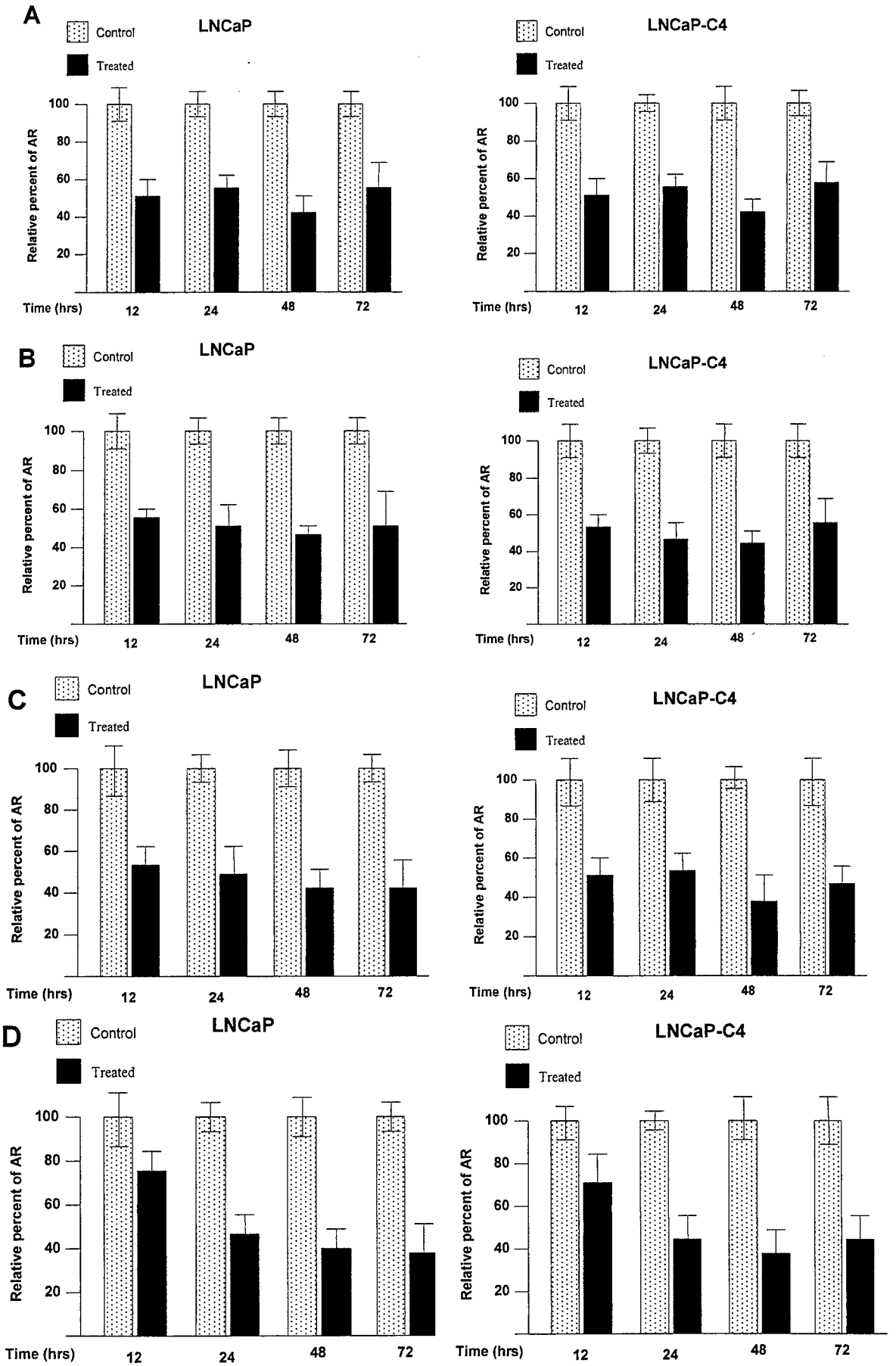


Figure 1. Expression of FGP in LNCaP and LNCaP-C4 cells. Magnification x 10

Secondly, we have detected biological effects of these RNA enzymes on endogenous expression of AR and AR-regulated genes, PSA and kallikrein 4 (KLK4) at different time points in these two transfected FGP cancer cell lines using quantitative real time PCR. These results showed that four different RNA enzymes were able to inhibit expression of endogenous AR gene in both the two cell lines (**Figure 2A-D**). The highest effect of these enzymes was at 48 hours after transfection Furthermore, multiple RNA enzymes had higher effects on inhibiting expression of AR compared to the individual anti-AR-enzyme (**Figure 2E**). Biological effects of these RNA enzymes had dose-dependent at 24-, 48- and 72-hour time periods (**Figure 2 F**). Also, we further analyzed AR-regulated genes, PSA and KLK4. **Figures 3** and **4** showed the same results with AR genes. These results were represented at 13th Annual Cancer Research Symposium, November, 2004, San Antonio, Texas (see appendix).


A
Control
Treated
LNCaP
LNCaP-C4
Relative percent of AR
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40
20
Time (hrs)
12
24
48
72
B
C
D

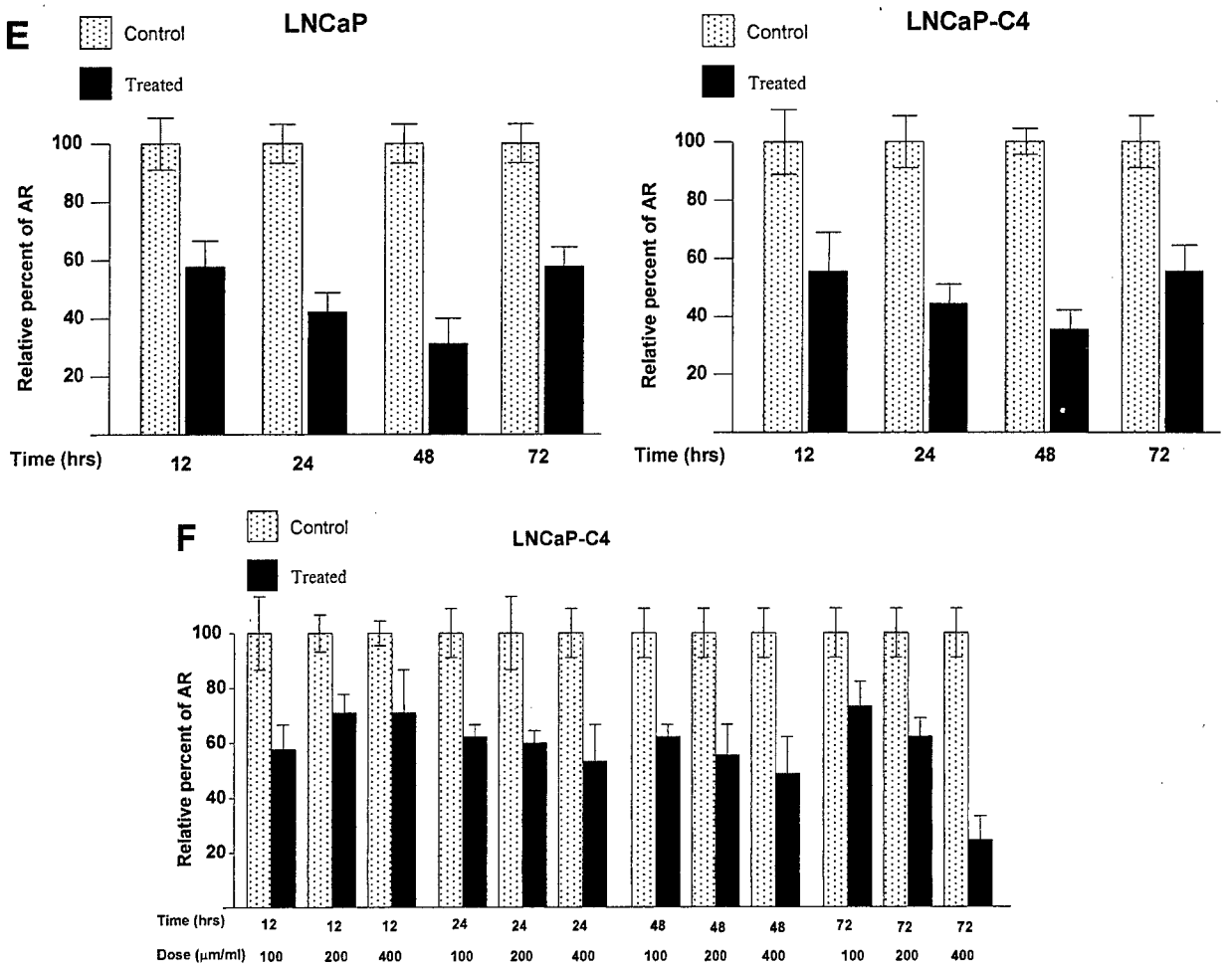
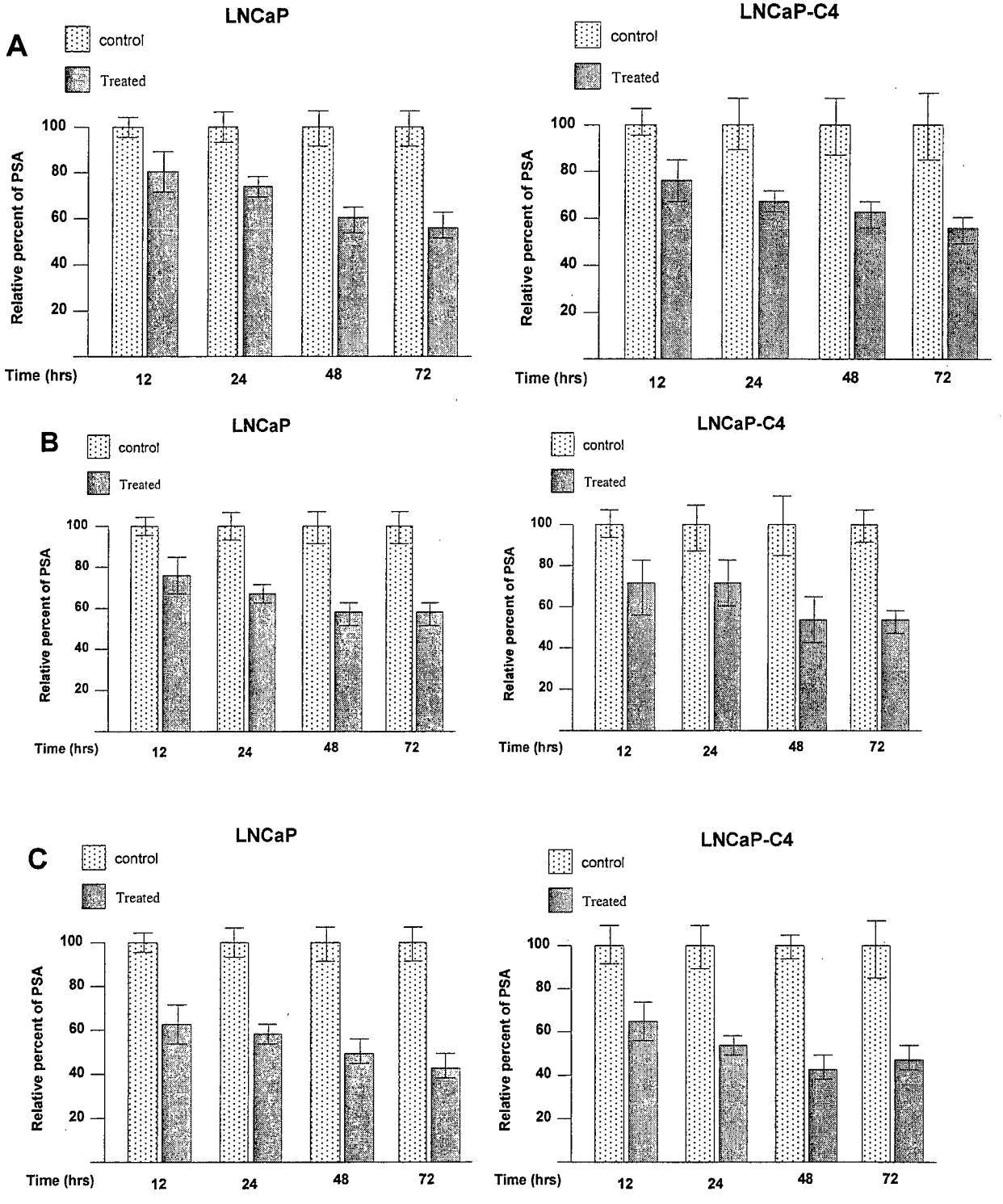


Figure 2. Biological effects of anti-AR enzymes on AR gene expression in human prostate cancer cells. 100 µm/l of individual or multiple anti-AR RNA enzymes or negative control (Ambion, Austin, TX) was transfected into the two human prostate cancer cells, respectively, according to the protocol provided by the manufacture (Ambion). After different time points, total RNA was isolated and cDNA was reversely transcribed. 100 ng of the transcribed cDNA were used for quantitative real time PCR. Amplification reactions were analyzed in real-time on an ABI 7500 (Applied Biosystems, Foster City, CA) using SYBR Green chemistry and the threshold values were calculated using SDS2 software (Applied Biosystems). Thermal cycling parameters were 95°C for 30s and 60°C for 1 min, 40 cycles. Reactions were performed in quadruplicate and threshold cycle numbers were averaged. A single melt curve peak was observed for each sample used in data analysis, confirming the purity and specificity of all amplified products. The threshold data generated was normalized to cyclophilin A. Primers used for AR and cyclophilin gene amplifications were as follows: cyclophilin; forward 5' ggtgacttcacacgc cataa 3'. reverse 5' catggcctccacaatattca 3'; AR: forward 5' ggccaggaaagcgacttcac 3' reversed 5'gacacaagtgggactgggatagg 3'. A, anti-AR RNA enzyme 1; B, anti-AR RNA enzyme 2; C, anti-AR RNA enzyme 3; D, anti-AR RNA enzyme 4; E, four anti-AR enzyme combination; F, different doses of anti-AR enzyme 3.


A
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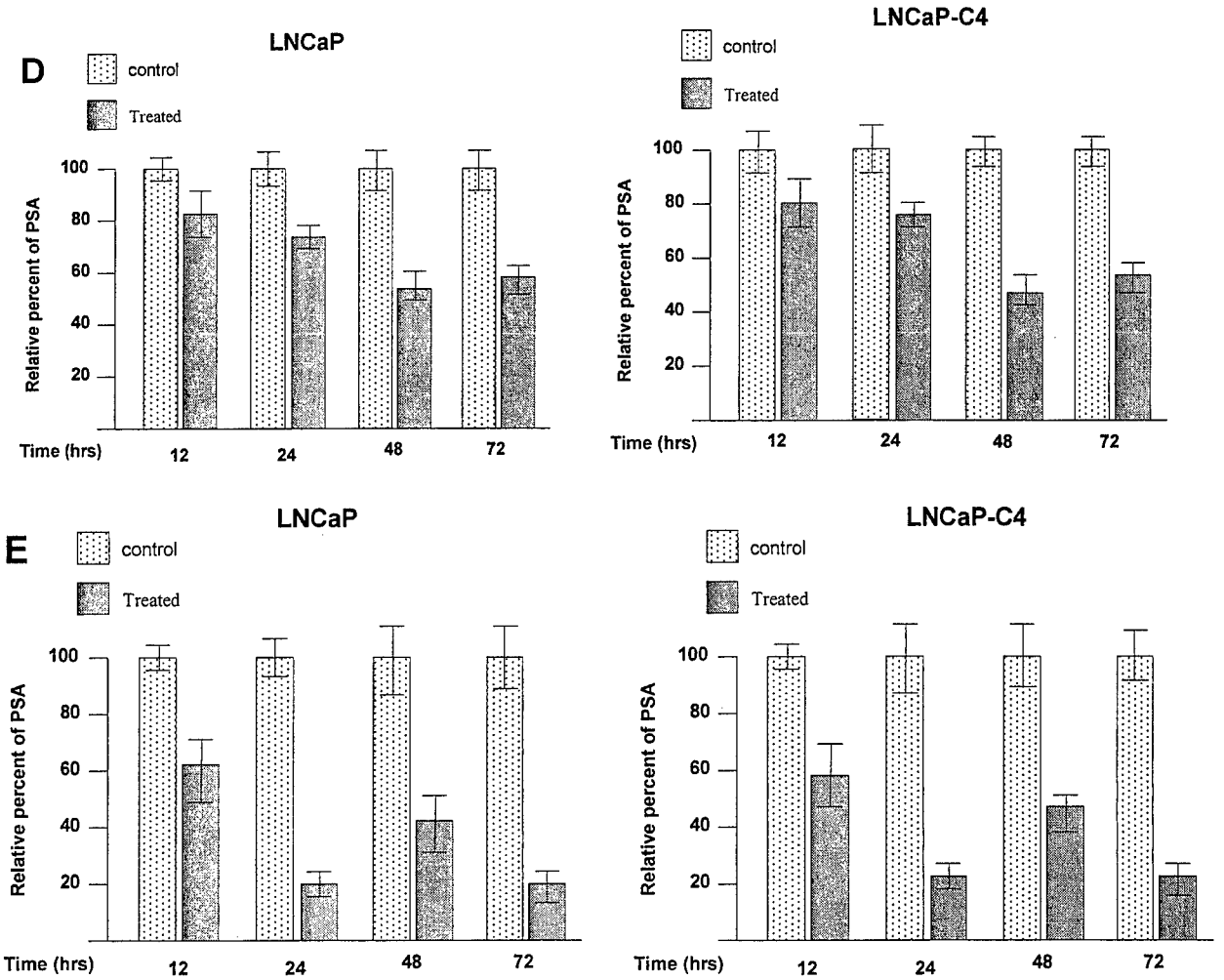
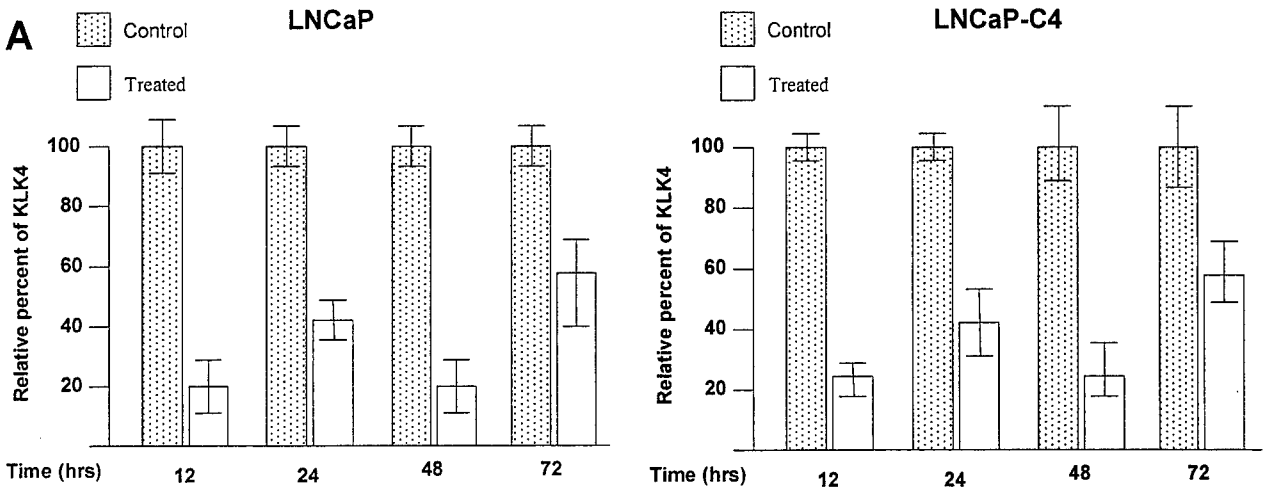
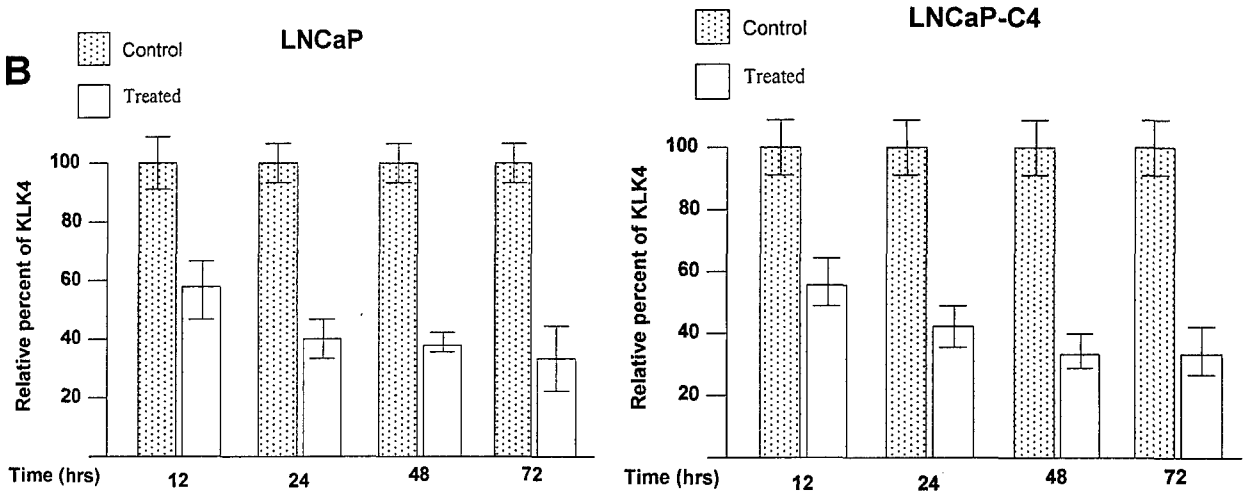


Figure 3 Biological functions of anti-AR enzymes on PSA gene expression in human prostate cancer cells. Experimental procedures were described above in Figure 2. Primers used for PAS gene amplification were as follows: forward 5'gagccaaggagggagggtctt3 ', reverse 5' tccccccatagtgaatcagctt 3'. A, anti-AR RNA enzyme 1; B, anti-AR RNA enzyme 2; C, anti-AR RNA enzyme 3; D, anti-AR RNA enzyme 4; E, four anti-AR enzyme combination.




B
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LNCaP-C4
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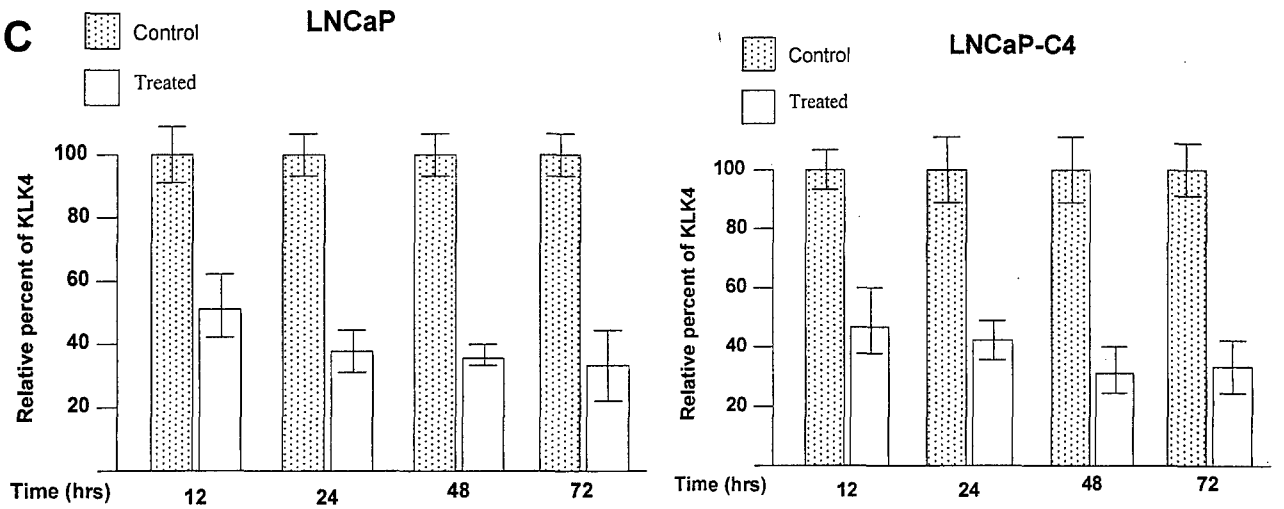
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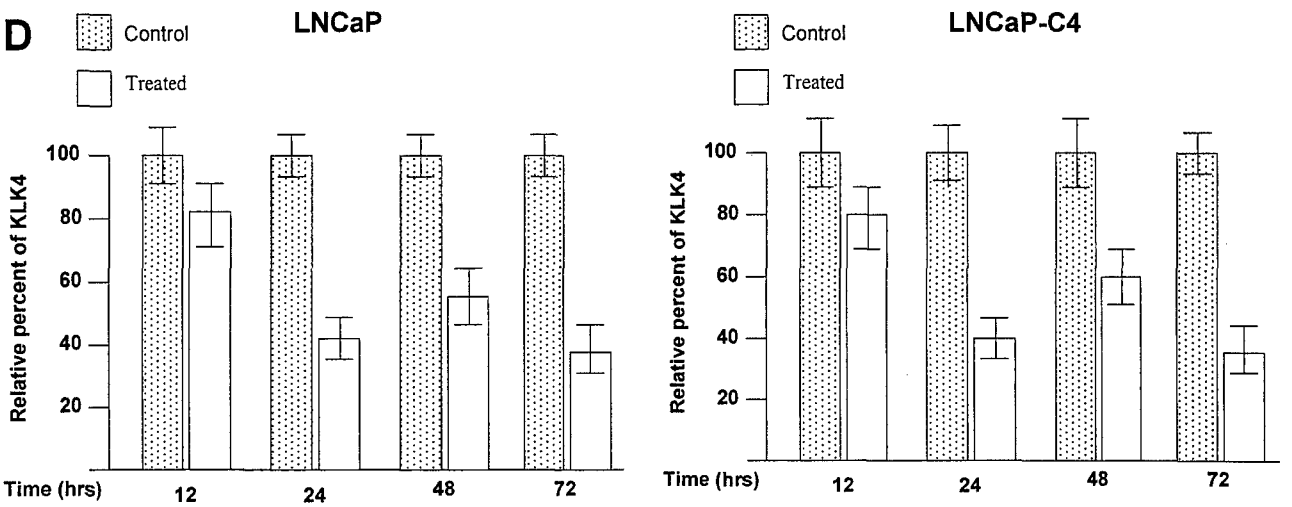
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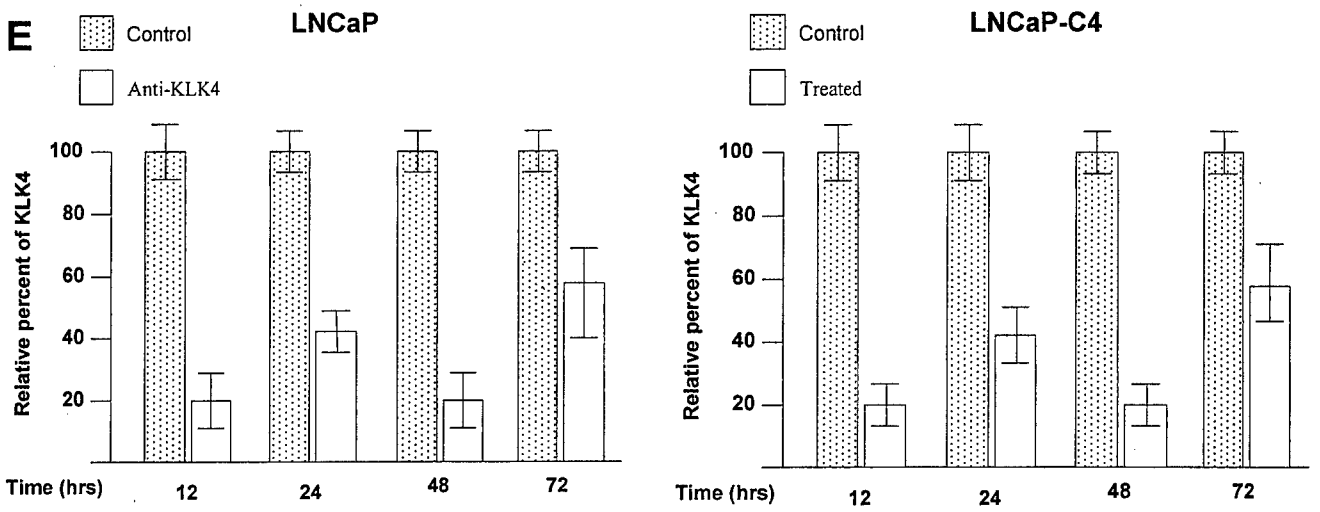

Figure 4 Biological functions of anti-AR enzymes on KLK4 gene expression in human prostate cancer cells. Experimental procedures were described above in Figure 2. Primers used for KLK4 gene amplification were as follows: forward 5' cagacccttgctcgctaacg 3', reverse 5' gctccggatggtgtcagact 3'. A, anti-AR RNA enzyme 1; B, anti-AR RNA enzyme 2; C, anti-AR RNA enzyme 3; D, anti-AR RNA enzyme 4; E, four anti-AR enzyme combination.

Key research accomplishments

1. Two FGP prostate cancer cell lines were established.
2. Four designed RNA enzymes were able to inhibit endogenous AR and AR-regulated gene expressions in both two human prostate cell lines.

Reportable Outcomes

In this project, we have established two FGP prostate cancer cell lines. These tumor cell lines are being implanted into immuno-deficiency nude mice. Also, four designed anti-AR RNA enzymes were able to inhibit AR and its related gene expressions at different time points in both two cancer cells. In vivo biological functions of these enzymes are being processed.

Conclusions

In this annual report, we demonstrated that designed anti-AR RNA enzymes are capable of inhibiting expression of AR, PAS and KLK4 genes in two human prostate cells.

References

1, Mangelsdorf, DJ., Thummel, C., Beato, M., Herrlich, P., Schutz, G., Umesono, K., Blumberg, B., Kastner, P., Mark, M. Chambon, P., et al (1995). The nuclear receptor superfamily: the second decade. Cell. 83(6):835-9.

2, Gregory, CW., Hamil, KG., Kim, D., Hall, SH., Pretlow, TG., Mohler, JL., French, FS. (1998). Androgen receptor expression in androgen-independent prostate cancer is associated with increased expression of androgen-regulated genes. Cancer Res. 58(24):5718-24.

3, Stanbrough, M., Leav, I., Kwan, PW., Bubley, GJ., Balk, SP. (2001). Prostatic intraepithelial neoplasia in mice expressing an androgen receptor transgene in prostate epithelium. Proc. Natl. Acad. Sci. USA 98(19):10823-8.

4, Gittes, RF. (1991). Carcinoma of the prostate. N. Engl. J. Med. 324(4):236-45.

5, Linja, MJ., Savinainen, KJ., Saramaki, OR., Tammela, TL., Vessella, RL., Visakorpi, T. (2001). Amplification and overexpression of androgen receptor gene in hormone-refractory prostate cancer. Cancer Res. 61(9):3550-5.

6, Grossmann, ME., Huang, H., Tindall, DJ.(2001). Androgen receptor signaling in androgen-refractory prostate cancer. J. Natl.Cancer Institute. 93(22):1687-97.

7, Chen, CD., Welsbie, DS., Tran, C., Baek, SH., Chen,R., Vessella, R., Rosenfeld, MG., Sawyers, CL. (2004). Molecular determinants of resistance to antiandrogen therapy. Nat. Medicine. 10(1):33-9.

8, Isaacs, JT., Isaacs, WB. (2004). Androgen receptor outwits prostate cancer drugs.[comment]. Nat. Medicine. 10(1):26-7.

9, Catalona, WJ. (1994). Management of cancer of the prostate. New Engl. J. of Med. 331(15):996-1004.

10, Chen, S., Song, CS., Lavrovsky, Y., Bi, B., Vellanoweth, R., Chatterjee, B., Roy, AK. (1998). Catalytic cleavage of the androgen receptor messenger RNA and functional inhibition of androgen receptor activity by a hammerhead ribozyme. Mol Endocrinol. 12(10):1558-66.

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Abstract #_____ Page #_____

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Type abstract in space below (see Abstract Instructions)

Regulation of Androgen Receptor Expression in Human Prostate Cancer Cells by a Novel Transcription Factor and Small Interference RNA. *Chen S, Wu, Y, Chuang, H, MacDougall M. The University of Texas Health Science Center at San Antonio, TX 78229, San Antonio, TX.

Androgen Receptor (AR) functions as a ligand-activated transcription factor for androgen-regulated genes. AR amplification and mutations play the critical roles in recurrence and metastasis of certain prostate cancers. **Objective:** First to investigate regulatory elements of AR gene in human prostate cancer versus normal cells; second to examine small interference RNA (siRNA) specific for AR gene in inhibiting AR expression in human cancer cells. **Methods:** Various constructs of human AR promoter-luciferase reporter gene were transfected into human prostate cancer (LN-cap) and normal (RWPE) cells, and luciferase activity was analyzed. A critical responsible element was identified and binding of nuclear protein(s) to this element was examined by electrophoretic mobility shift assay (EMSA). In addition, inhibition of AR expression by the siRNA was determined by quantitative real time PCR and Western blotting assays. **Results:** We characterized that one element in human AR promoter was responsible for AR gene expression. Mutations of this element resulted in a 2- and 3-fold decline of promoter activity in RWPE and LN-cap cells. A series of mutation and competition assays showed that the element interacts with a novel nuclear protein by EMSA and expression levels of this nuclear protein was higher in the cancer cells than that of normal cells. The consensus sequence of this element is conserved across species. In addition, the siRNA had effects on inhibiting AR gene expression in LN-cap cells and the highest efficiency at 12 hours after treatment. **Conclusions:** This study speculates that this element in AR gene promoter plays some distinct functions in regulating AR expression in human cancer versus normal cells. Furthermore, the siRNA provides a new avenue for inhibition of androgen action by selective mRNA degradation with its potential therapeutic application through targeted gene delivery vectors. This work was supported by DOD grant W81XWH and NIDR grant DE11658.

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